

FIG. 1

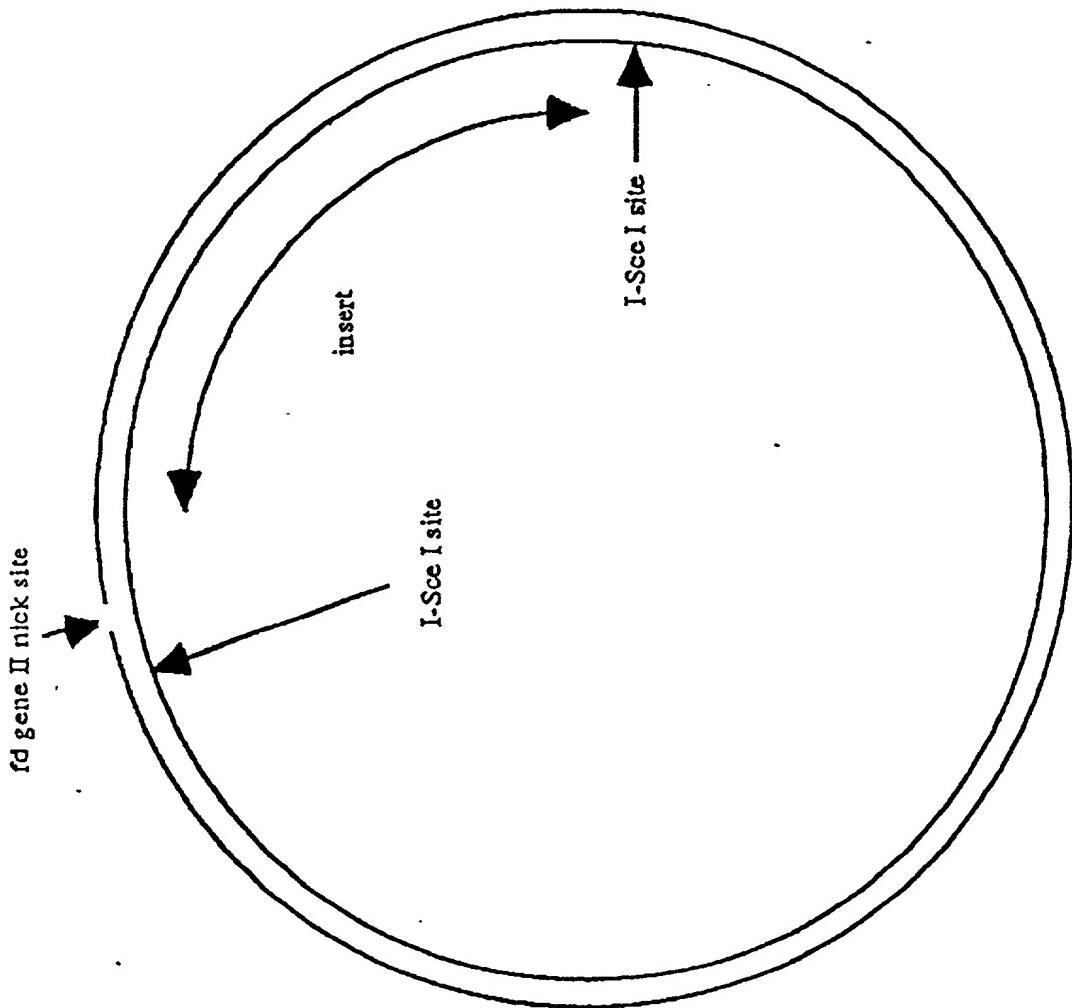


FIG. 2

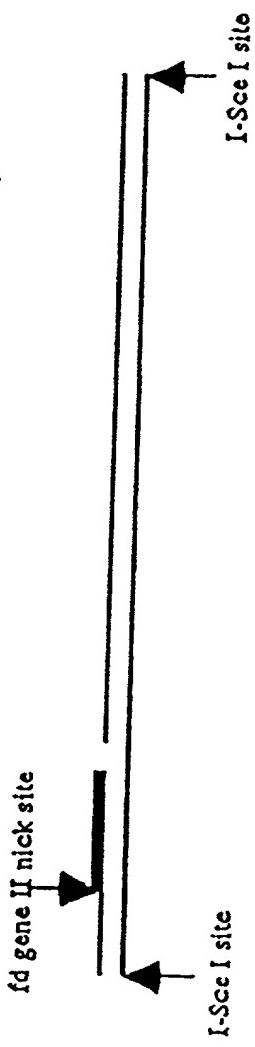
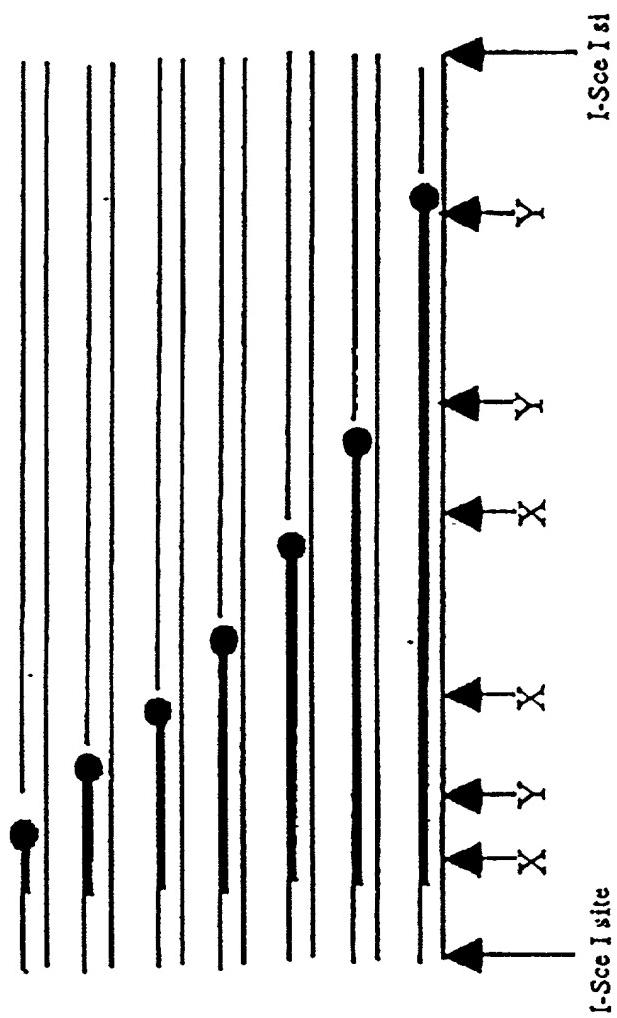


FIG. 3



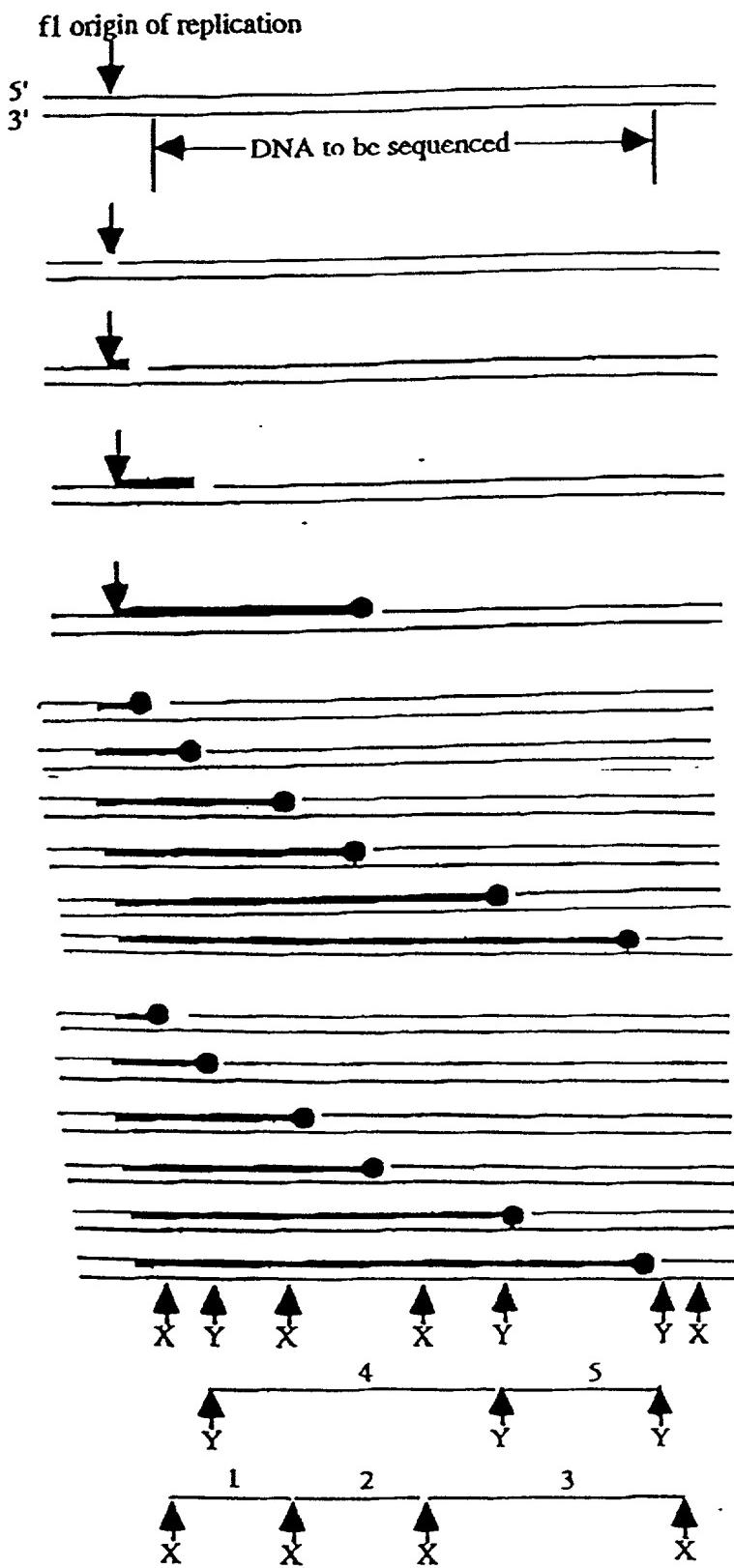


FIG. 4A

FIG. 4B

FIG. 4C

FIG. 4D

FIG. 4E

FIG. 4F

FIG. 4G

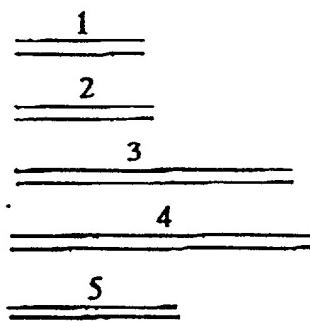


FIG. 4H

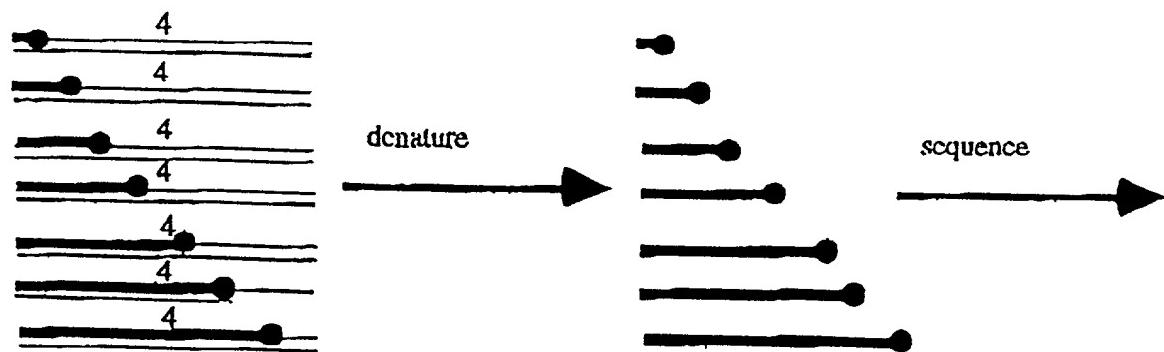


FIG. 4I

FIG. 5

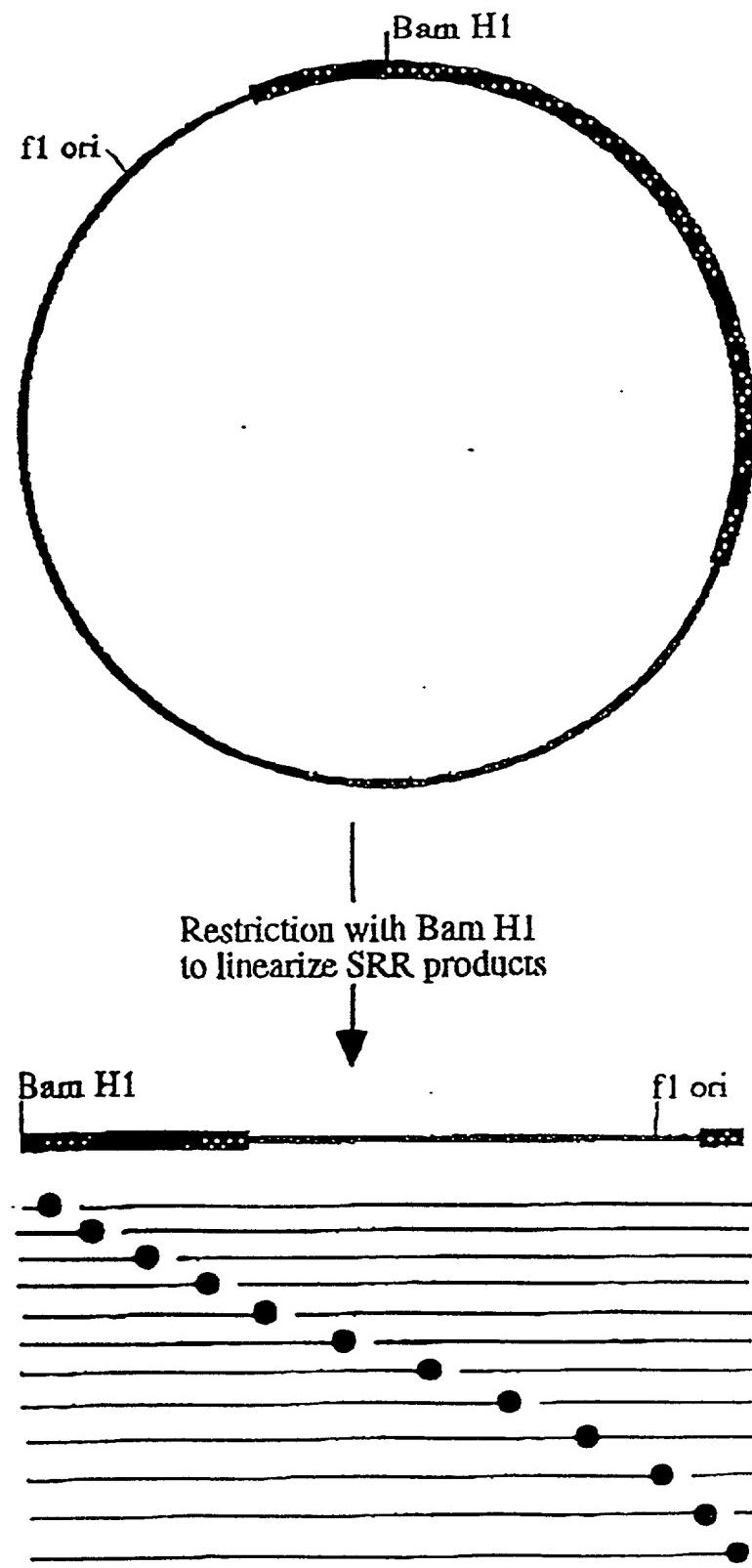
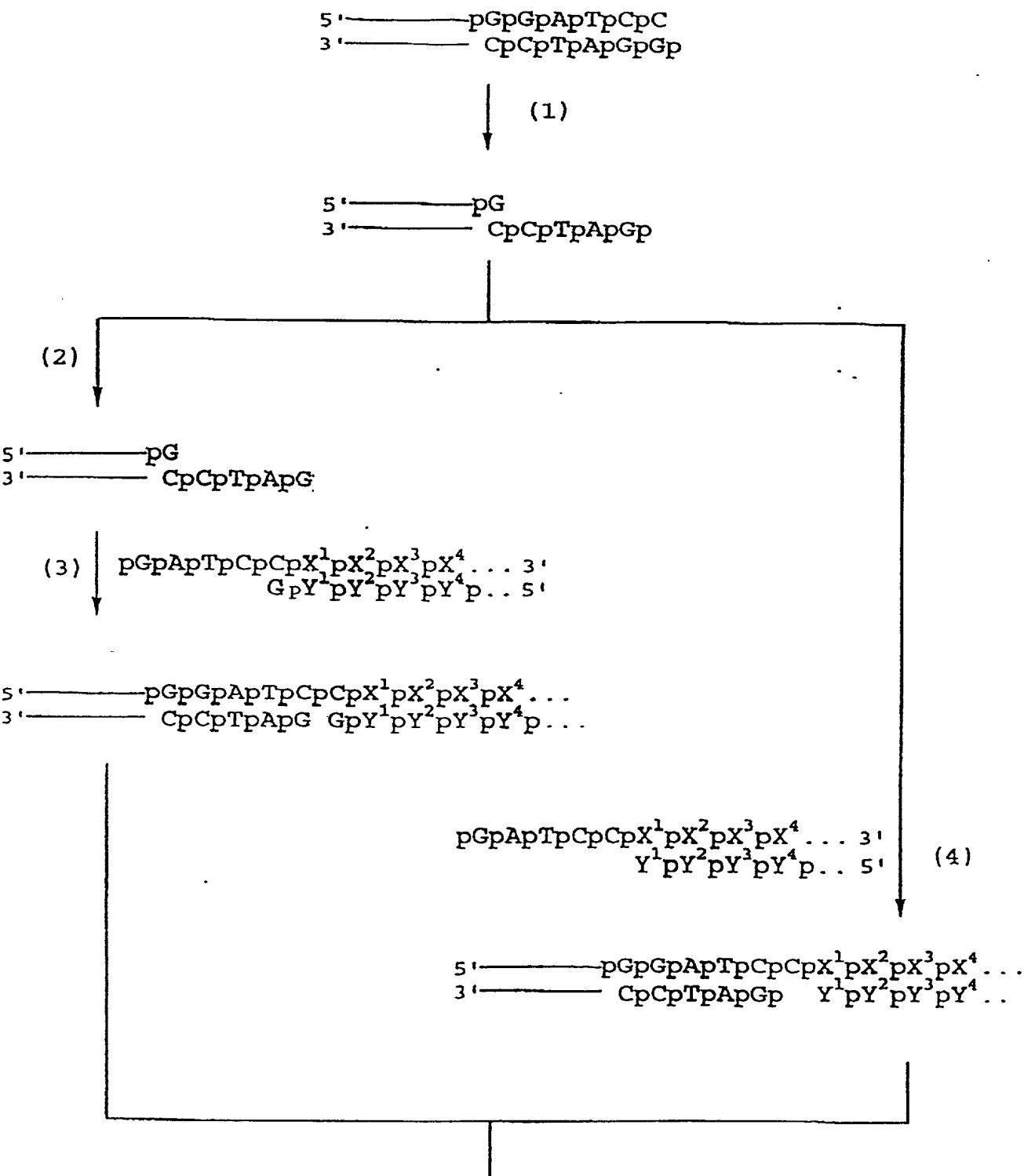


FIG. 6

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STRAND REPLACEMENT REACTION

FIG. 7A

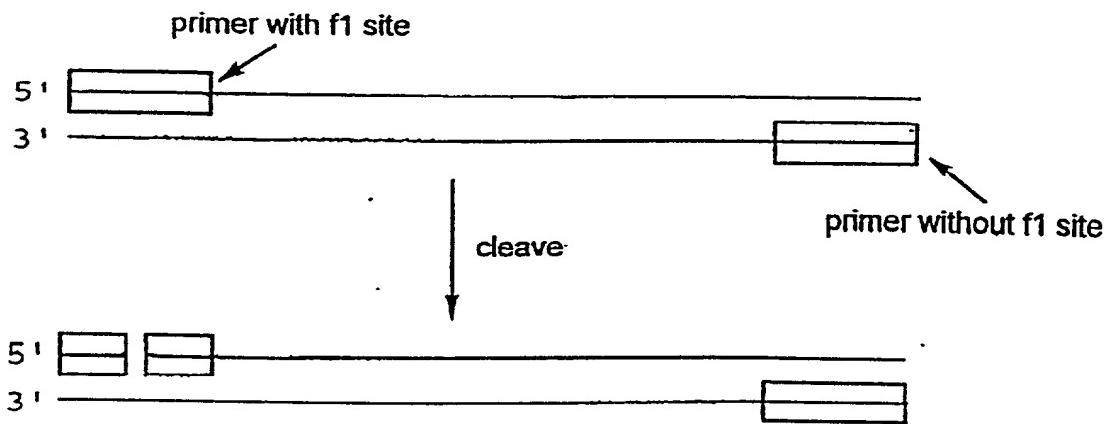


FIG. 7B

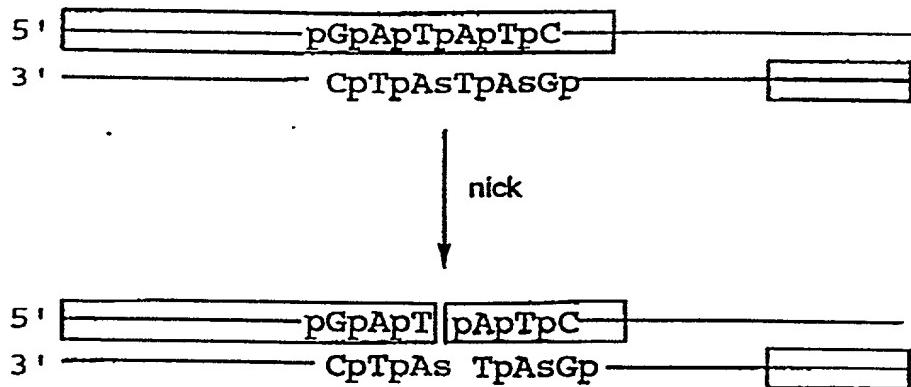


FIG. 7C

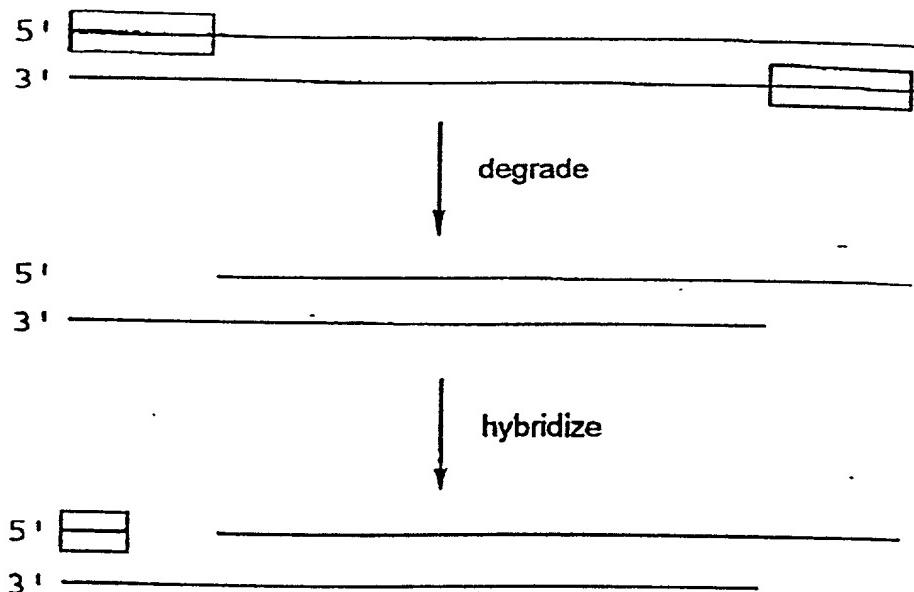
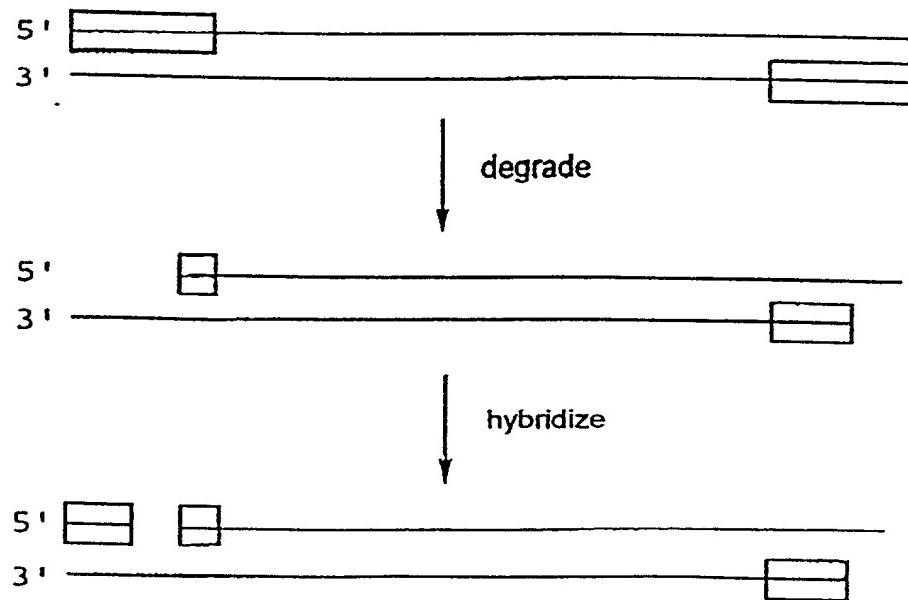


FIG. 7D



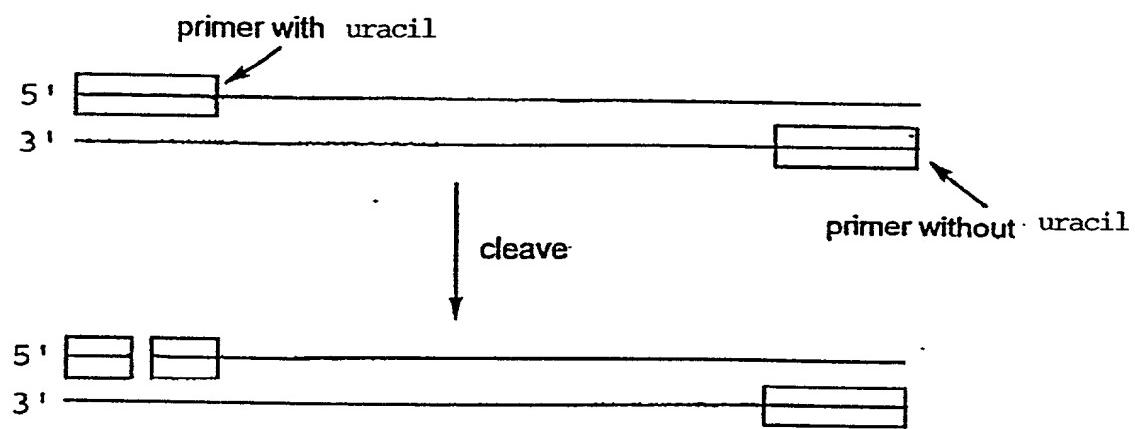


FIG. 7E

FIG. 8

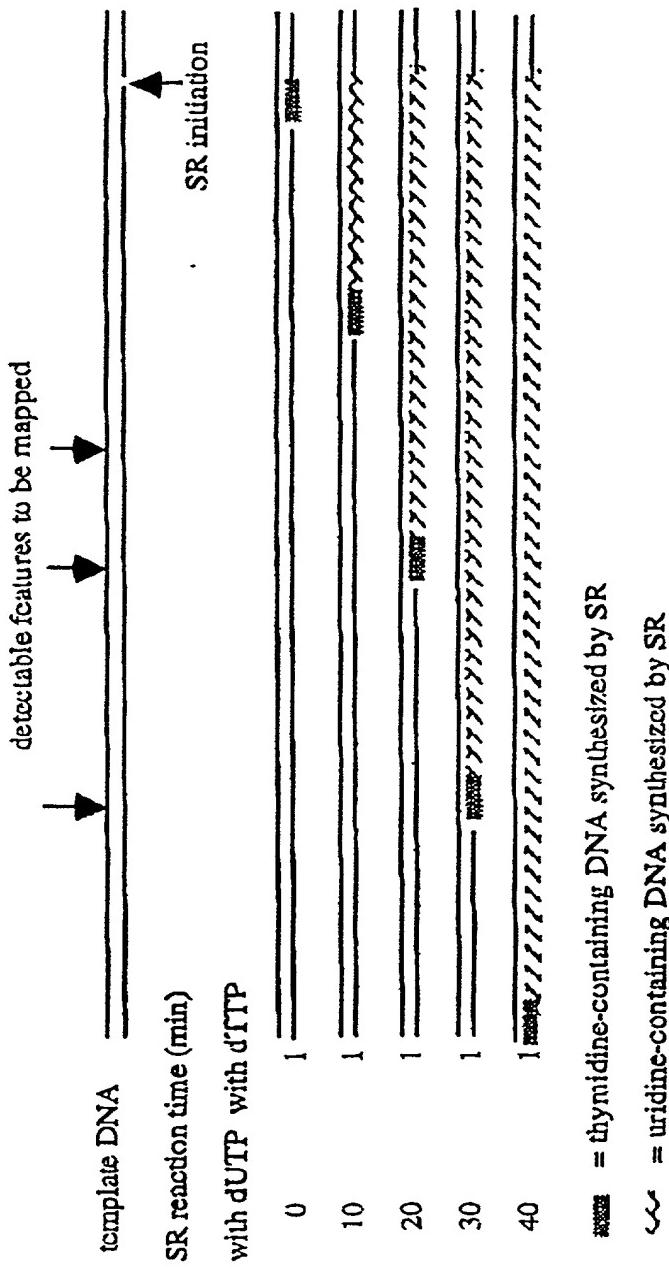


FIG. 9

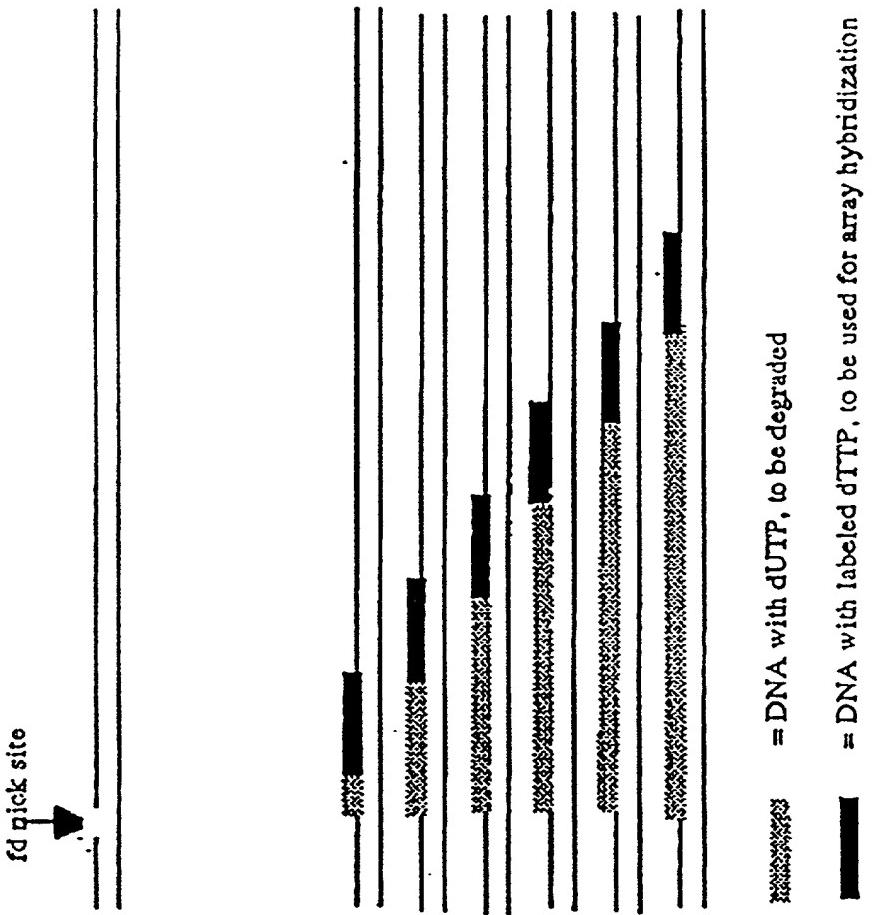


FIG. 10A

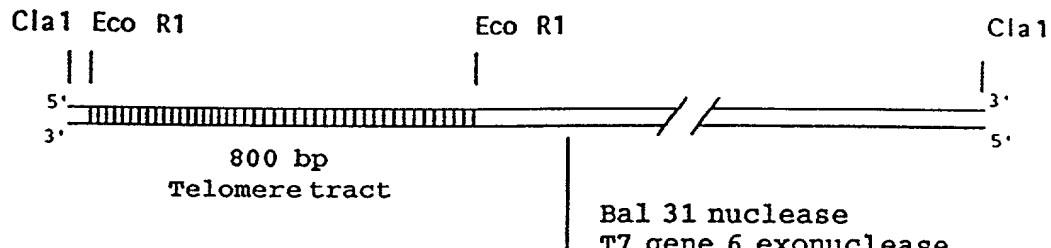


FIG. 10B

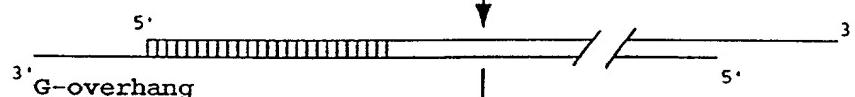


FIG. 10C

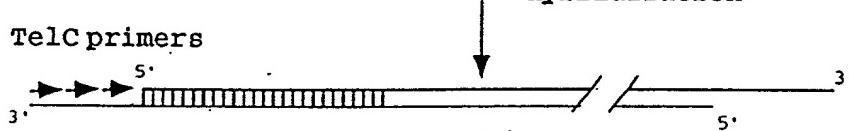


FIG. 10D

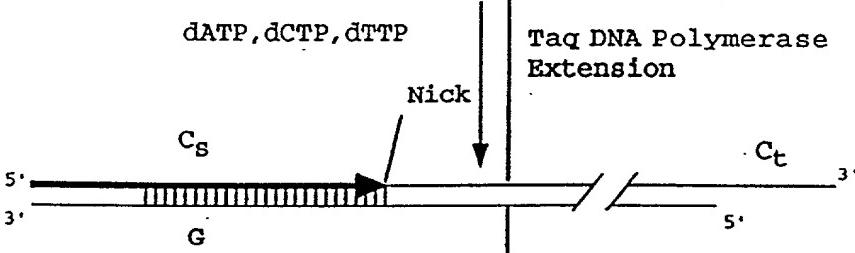


FIG. 10E

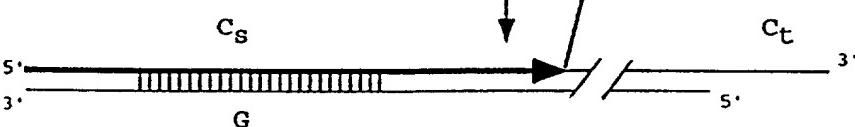


FIG. 11

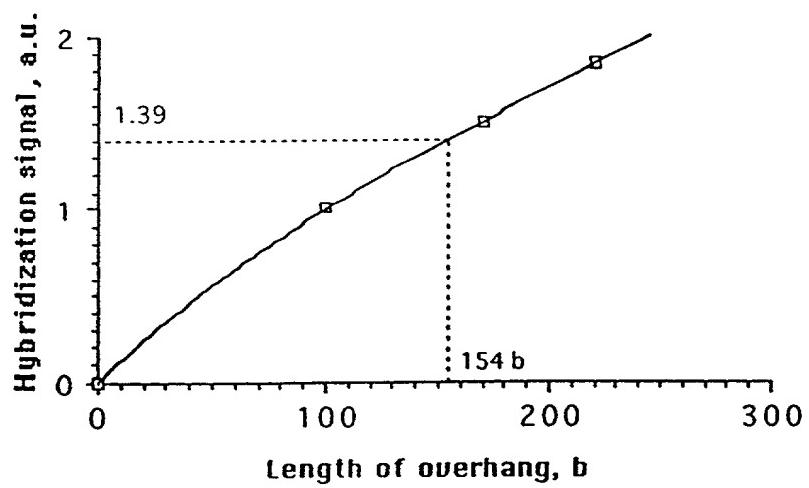


FIG. 12

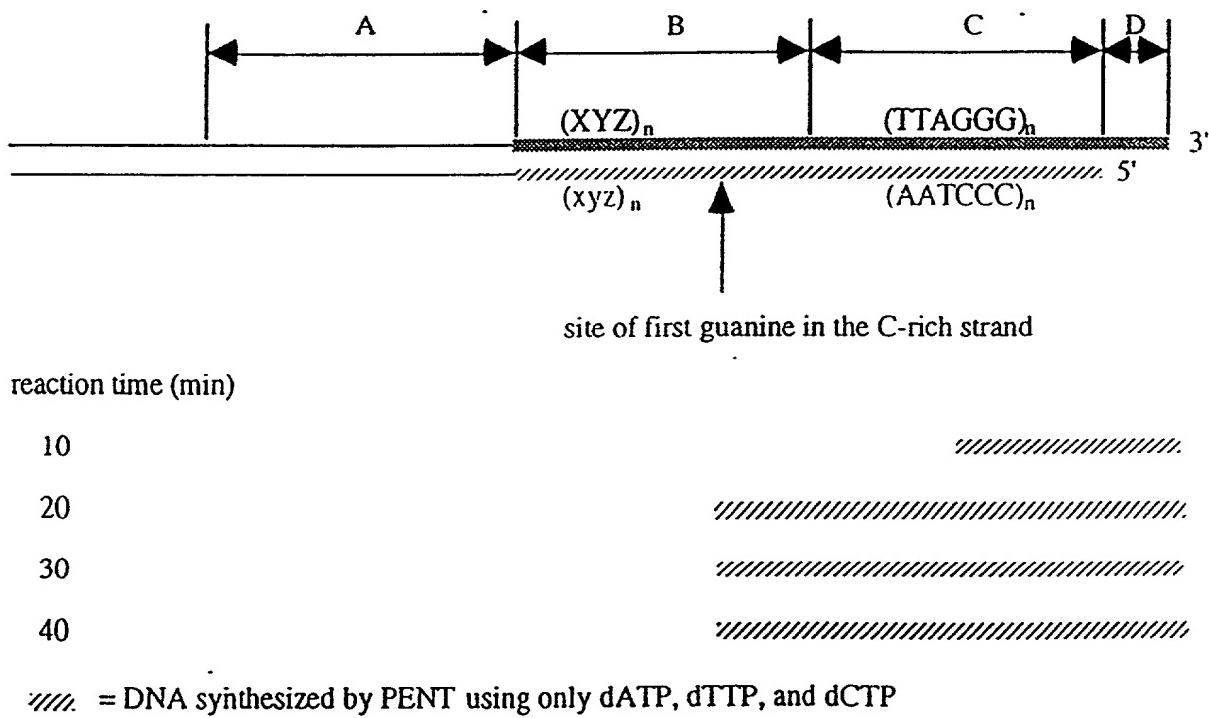


FIG. 13

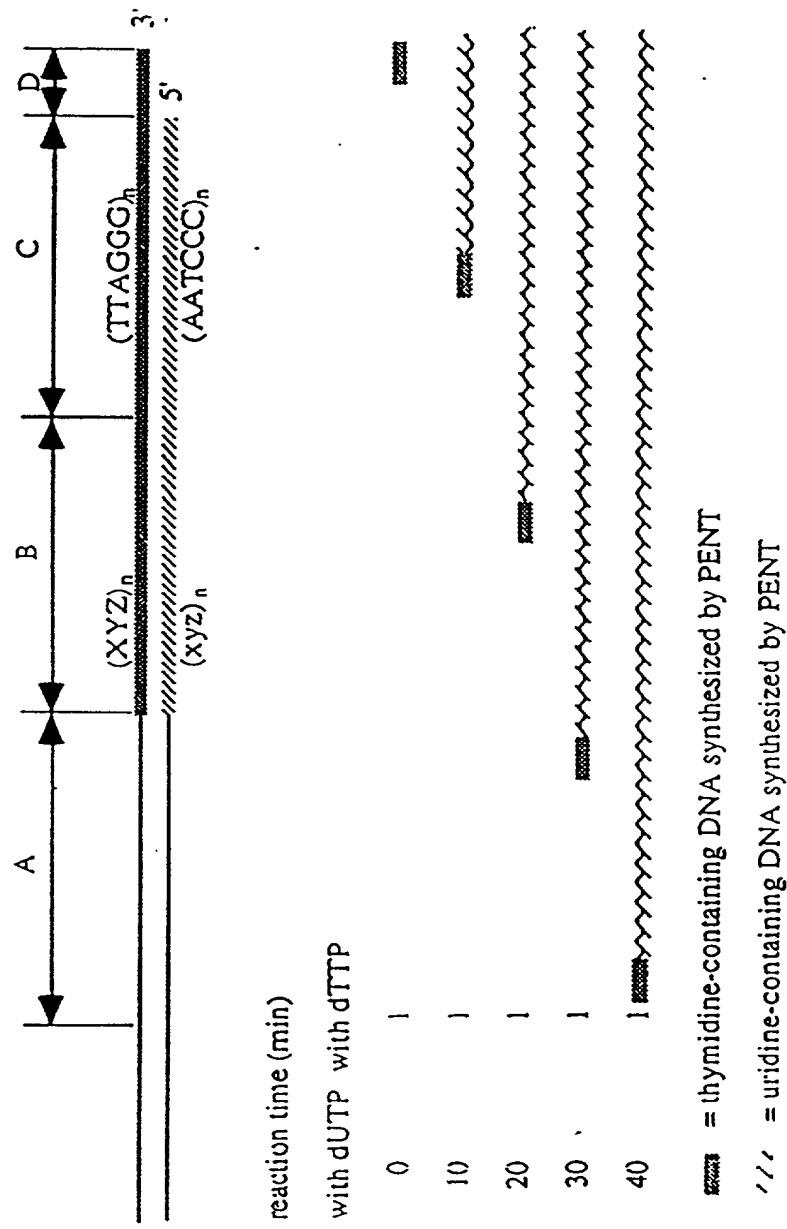


FIG. 14A

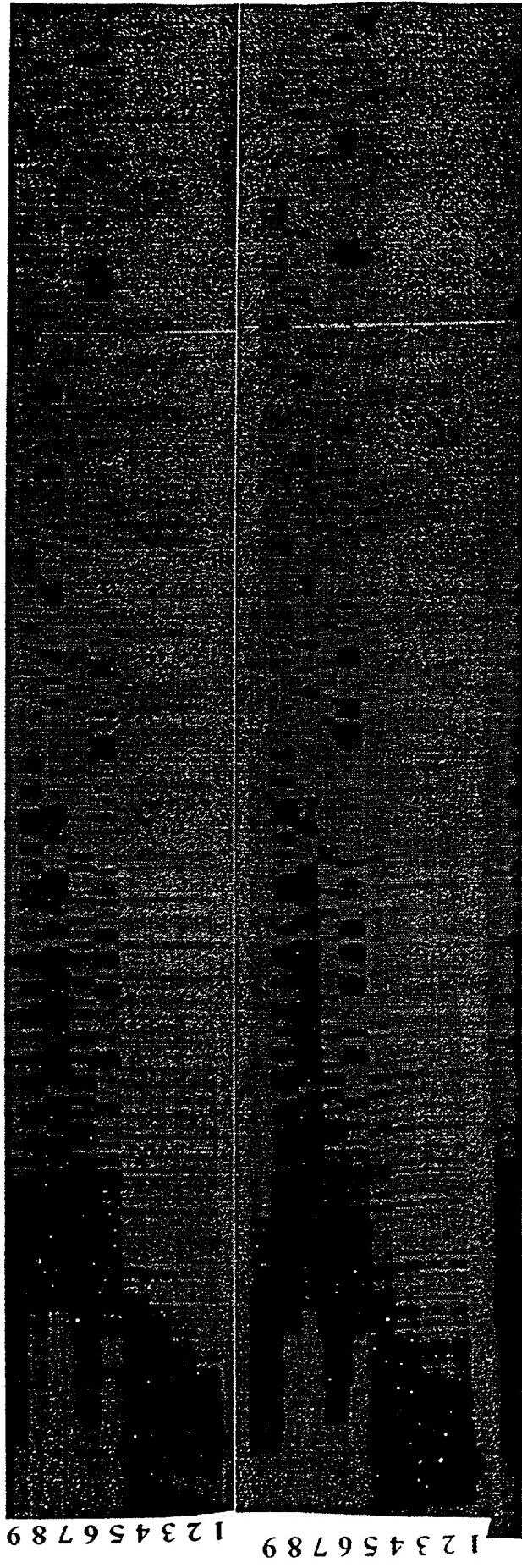
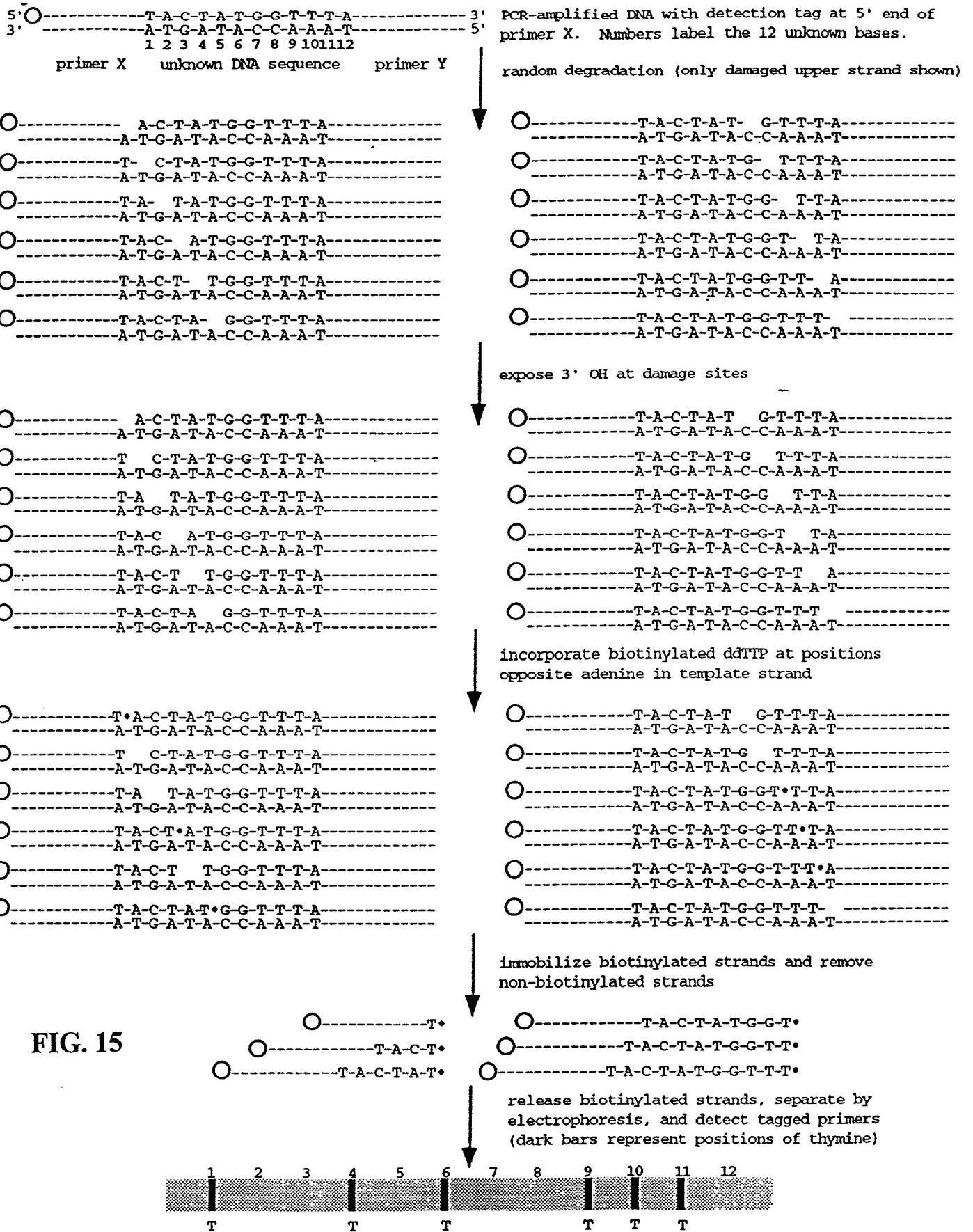
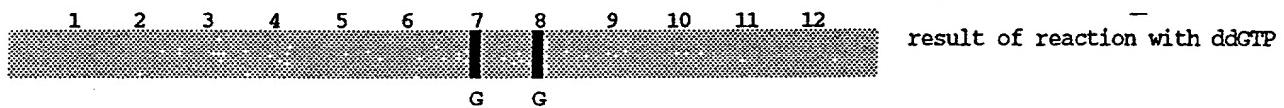
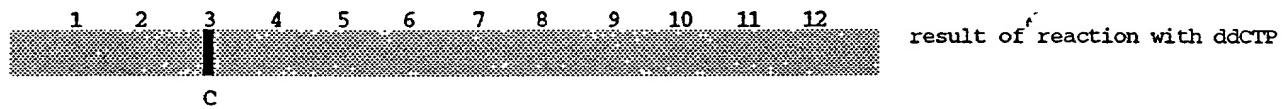
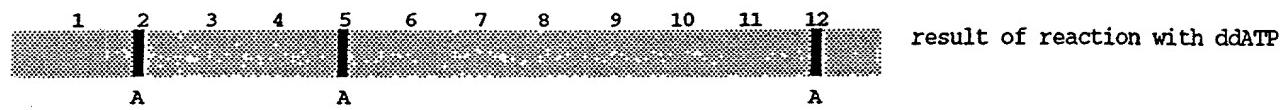
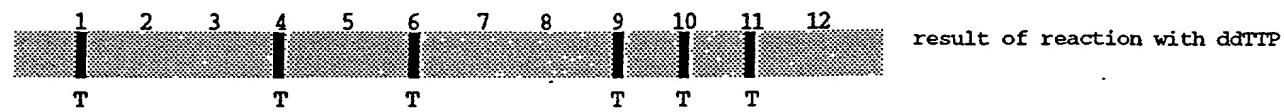


FIG. 14B



Results of size separation of detectable products of four ddNTP reactions



summation of ddNTP results into complete base sequence

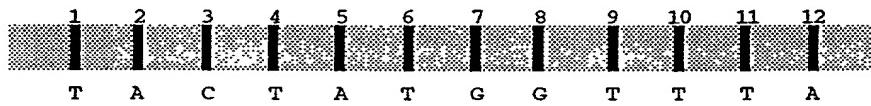
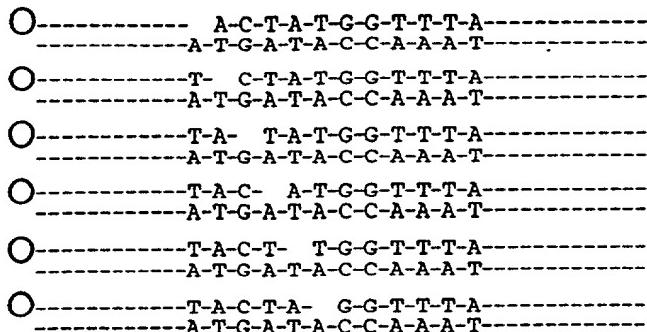


FIG. 16

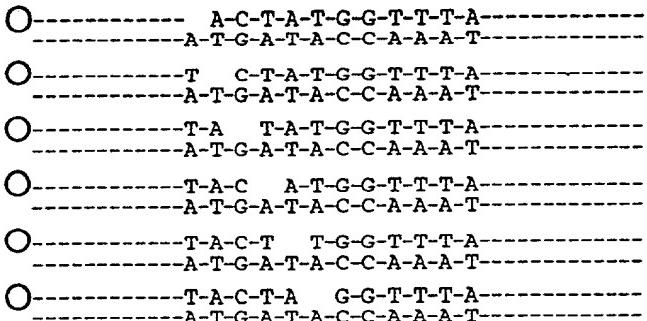
5' O-----T-A-C-T-A-T-G-G-T-T-T-A-----3'
 3' -----A-T-G-A-T-A-C-C-A-A-A-T-----5'
 1 2 3 4 5 6 7 8 9 10 11 12
 primer X unknown DNA sequence primer Y

PCR-amplified DNA with detection tag at 5' end of primer X. Numbers label the 12 unknown bases.

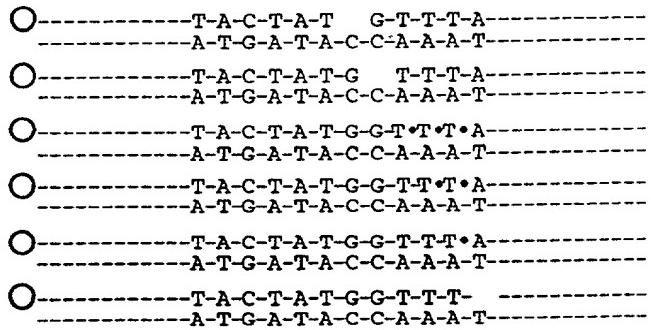
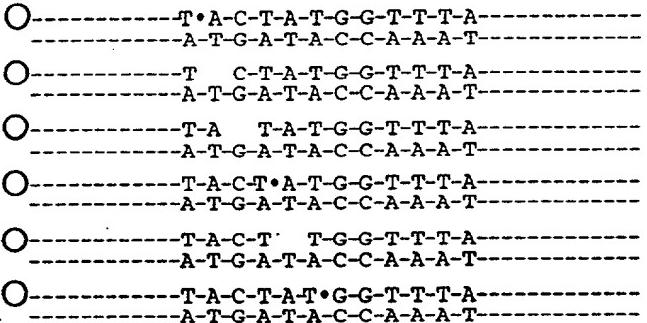
random degradation (only damaged upper strand shown)



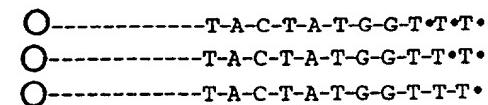
expose 3' OH at damage sites



incorporate biotinylated ddTTP at positions opposite adenine in template strand



immobilize biotinylated strands and remove non-biotinylated strands



release biotinylated strands, separate by electrophoresis, and detect tagged primers (dark bars represent positions of terminal thymine)

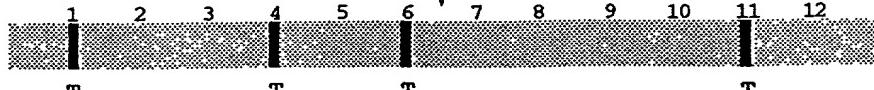
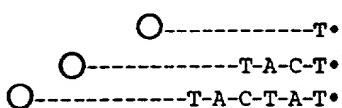
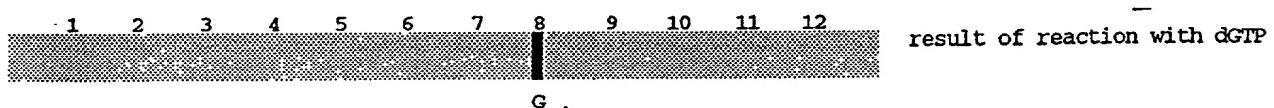
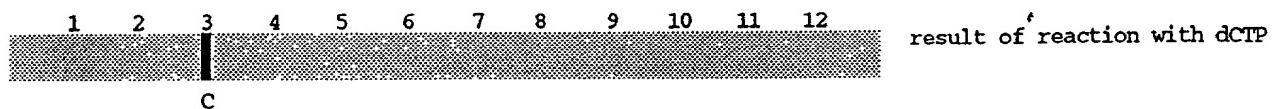
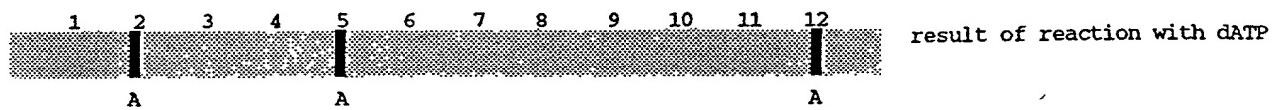
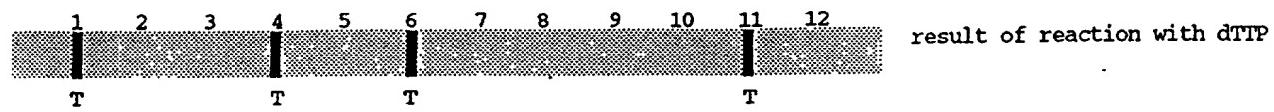


FIG. 17



Results of size separation of detectable products of four dNTP reactions



Summation of dNTP results into complete base sequence
(positions of bases in parentheses are inferred)

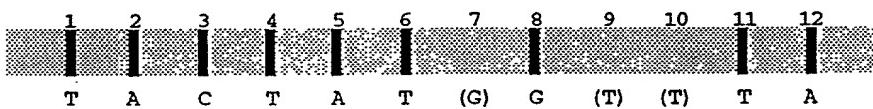
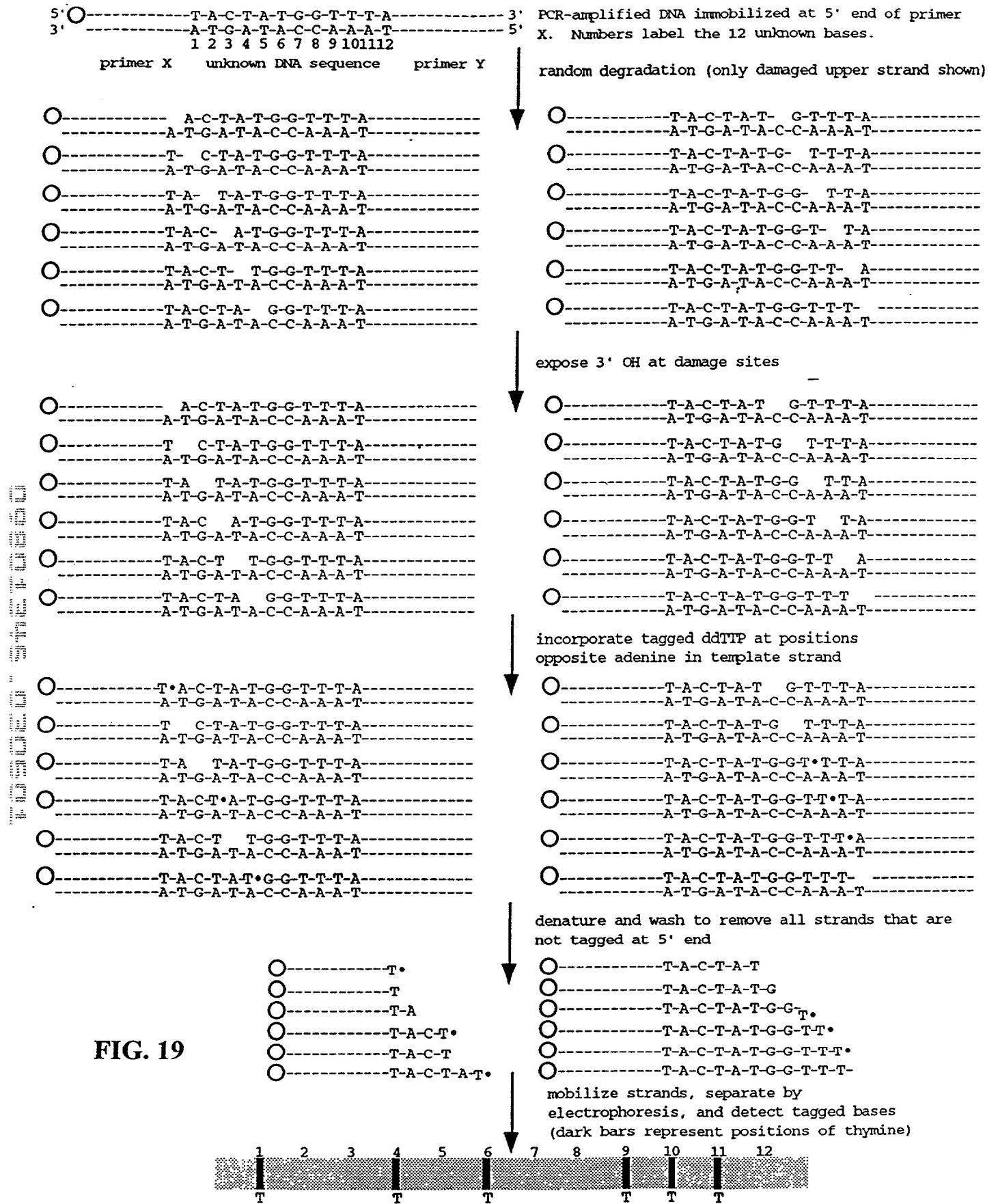
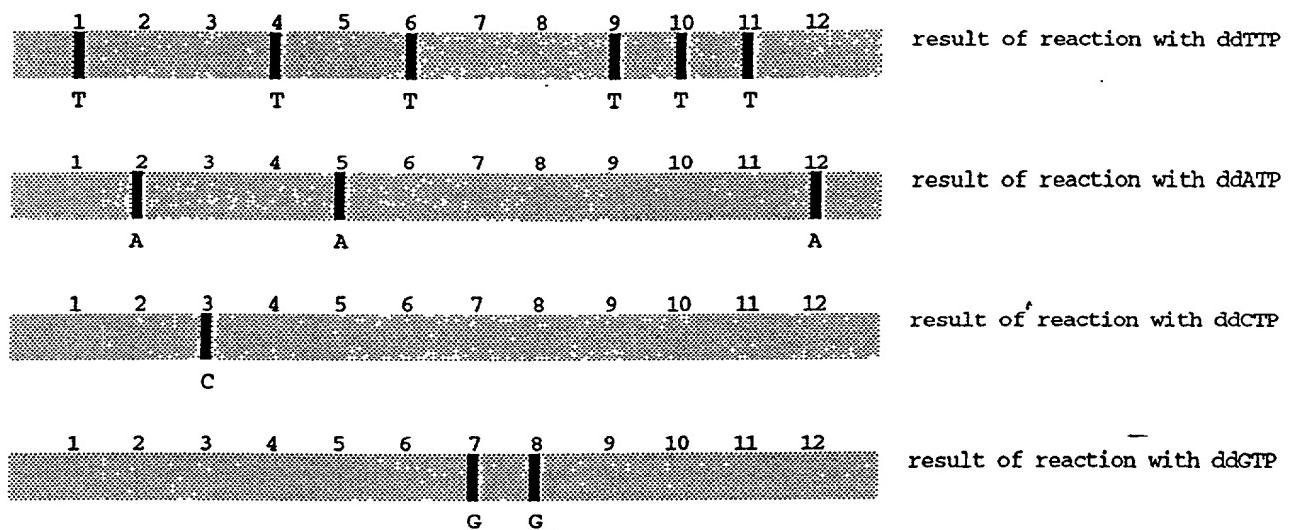


FIG. 18



Results of size separation of detectable products of four ddNTP reactions



summation of ddNTP results into complete base sequence

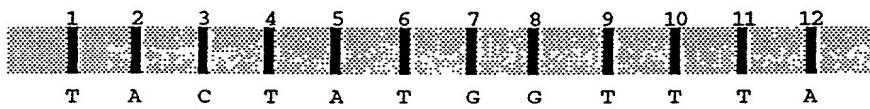


FIG. 20

PCR amplify, immobilize, and expose 3' OH
at random sites as in Fig. 5.

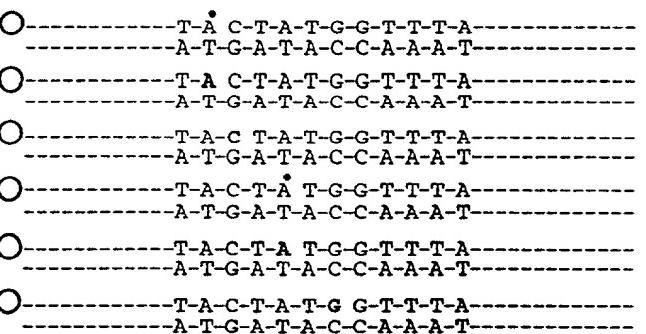
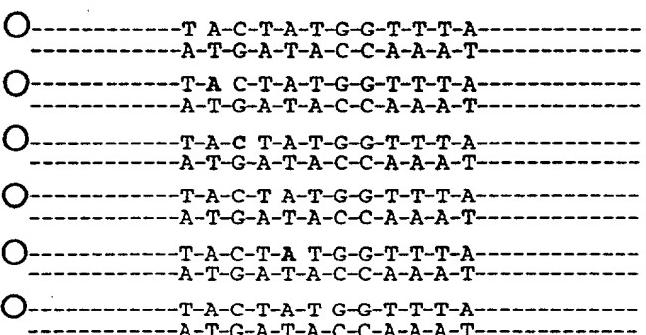
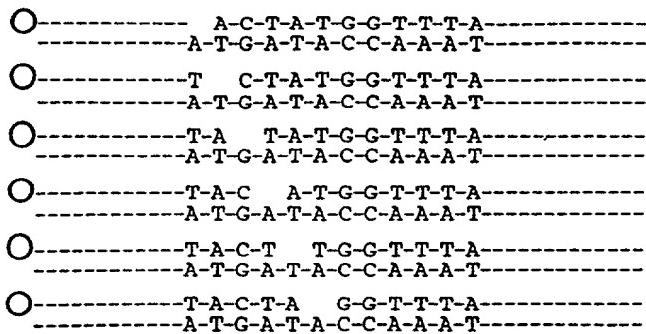
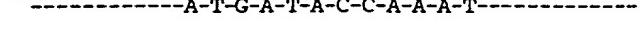
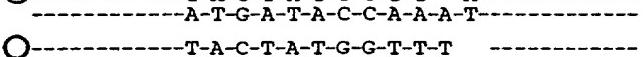
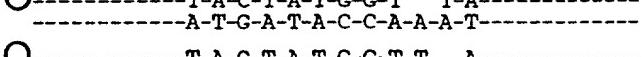
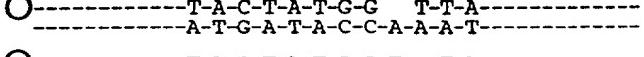
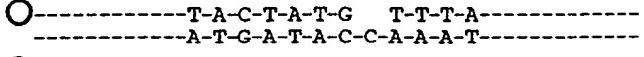
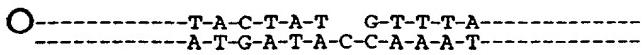
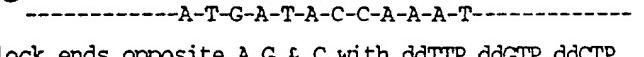
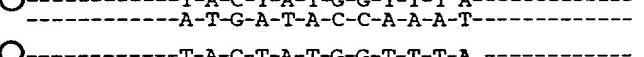
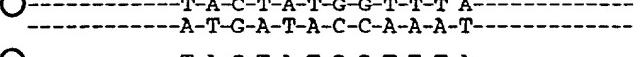
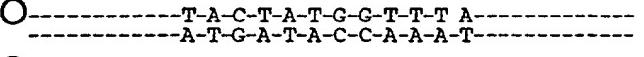
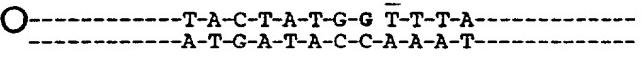
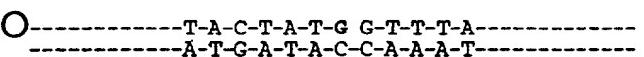


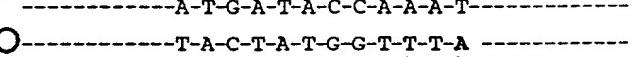
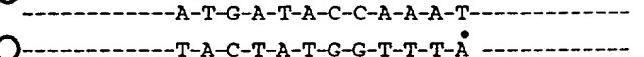
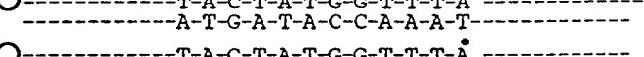
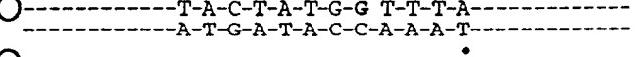
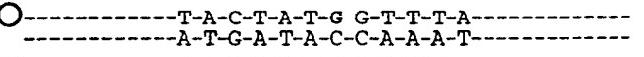
FIG. 21



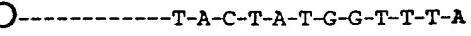
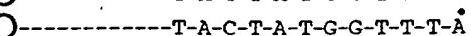
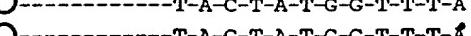
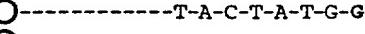
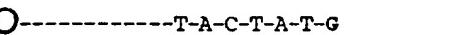
Block ends opposite T, G & C with ddATP, ddGTP, ddCTP
(shown in bold letters), remove ddNTPs, then add dTTP.



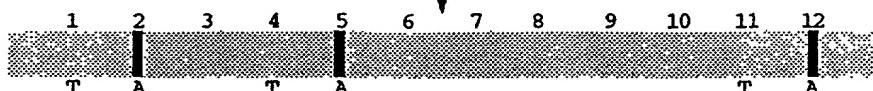
Block ends opposite A, G & C with ddTTP, ddGTP, ddCTP
(shown in bold letters), remove ddNTPs, then add
tagged ddATP.



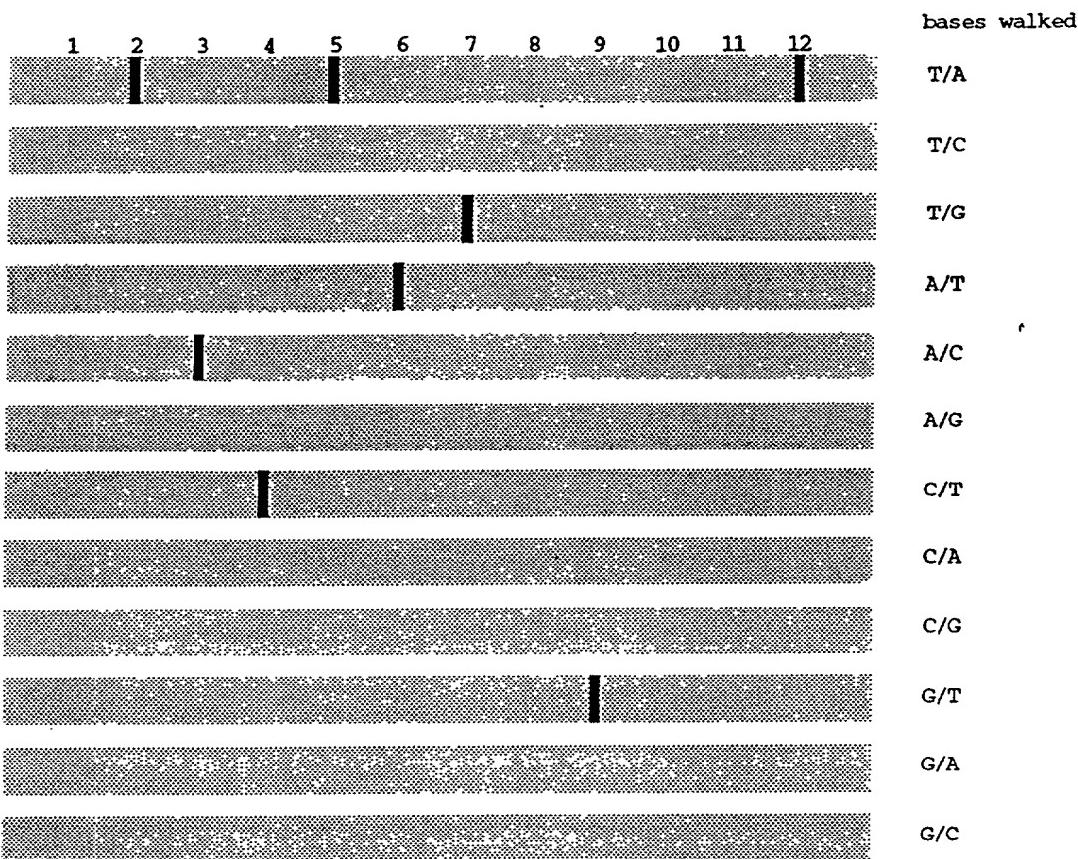
Denature and wash to remove all strands that are
not tagged at 5' end.



Mobilize strands, separate by electrophoresis,
and detect tagged bases (dark bars).



Size separation of the products of twelve 2-base walk reactions



Assembly of complete sequence from the results of individual reactions
(inferred bases in parentheses)



FIG. 22

PCR amplify, immobilize, and expose 3' OH at random sites as in Fig. 5.

○-----A-C-T-A-T-G-G-T-T-T-A
-----A-T-G-A-T-A-C-C-A-A-A-T
○-----T C-T-A-T-G-G-T-T-T-A
-----A-T-G-A-T-A-C-C-A-A-A-T
○-----T-A T-A-T-G-G-T-T-T-A
-----A-T-G-A-T-A-C-C-A-A-A-T
○-----T-A-C A-T-G-G-T-T-T-A
-----A-T-G-A-T-A-C-C-A-A-A-T
○-----T-A-C-T T-G-G-T-T-T-A
-----A-T-G-A-T-A-C-C-A-A-A-T
○-----T-A-C-T-A G-G-T-T-T-A
-----A-T-G-A-T-A-C-C-A-A-A-T

○-----T-A-C-T-A-T G-T-T-T-A
-----A-T-G-A-T-A-C-C-A-A-A-T
○-----T-A-C-T-A-T-G T-T-T-A
-----A-T-G-A-T-A-C-C-A-A-A-T
○-----T-A-C-T-A-T-G-G T-T-A
-----A-T-G-A-T-A-C-C-A-A-A-T
○-----T-A-C-T-A-T-G-G-T-T A
-----A-T-G-A-T-A-C-C-A-A-A-T
○-----T-A-C-T-A-T-G-G-T-T-T
-----A-T-G-A-T-A-C-C-A-A-A-T

Block ends opposite T,G & C with ddATP,ddGTP,ddCTP
(shown in bold letters); remove ddNTPs, then add dTTP.

○-----T A-C-T-A-T-G-G-T-T-T-A
-----A-T-G-A-T-A-C-C-A-A-A-T
○-----T-A C-T-A-T-G-G-T-T-T-A
-----A-T-G-A-T-A-C-C-A-A-A-T
○-----T-A-C T-A-T-G-G-T-T-T-A
-----A-T-G-A-T-A-C-C-A-A-A-T
○-----T-A-C-T A-T-G-G-T-T-T-A
-----A-T-G-A-T-A-C-C-A-A-A-T
○-----T-A-C-T-A T-G-G-T-T-T-A
-----A-T-G-A-T-A-C-C-A-A-A-T
○-----T-A-C-T-A-T G-G-T-T-T-A
-----A-T-G-A-T-A-C-C-A-A-A-T

○-----T-A-C-T-A-T-G G-T-T-T-A
-----A-T-G-A-T-A-C-C-A-A-A-T
○-----T-A-C-T-A-T-G-G T-T-T-A
-----A-T-G-A-T-A-C-C-A-A-A-T
○-----T-A-C-T-A-T-G-G-T-T-T A
-----A-T-G-A-T-A-C-C-A-A-A-T
○-----T-A-C-T-A-T-G-G-T-T-T A
-----A-T-G-A-T-A-C-C-A-A-A-T
○-----T-A-C-T-A-T-G-G-T-T-T
-----A-T-G-A-T-A-C-C-A-A-A-T

Block ends opposite A,G & C with ddTTP,ddGTP,ddCTP
(shown in bold), remove ddNTPs, then add dATP.

○-----T-A C-T-A-T-G-G-T-T-T-A
-----A-T-G-A-T-A-C-C-A-A-A-T
○-----T-A C-T-A-T-G-G-T-T-T-A
-----A-T-C-A-T-A-C-C-A-A-A-T
○-----T-A-C T-A-T-G-G-T-T-T-A
-----A-T-G-A-T-A-C-C-A-A-A-T
○-----T-A-C-T-A T-G-G-T-T-T-A
-----A-T-G-A-T-A-C-C-A-A-A-T
○-----T-A-C-T-A T-G-G-T-T-T-A
-----A-T-G-A-T-A-C-C-A-A-A-T
○-----T-A-C-T-A-T G G-T-T-T-A
-----A-T-G-A-T-A-C-C-A-A-A-T

○-----T-A-C-T-A-T-G G-T-T-T-A
-----A-T-G-A-T-A-C-C-A-A-A-T
○-----T-A-C-T-A-T-G-G T-T-T-A
-----A-T-G-A-T-A-C-C-A-A-A-T
○-----T-A-C-T-A-T-G-G-T-T-T A
-----A-T-G-A-T-A-C-C-A-A-A-T
○-----T-A-C-T-A-T-G-G-T-T-T A
-----A-T-G-A-T-A-C-C-A-A-A-T
○-----T-A-C-T-A-T-G-G-T-T-T
-----A-T-G-A-T-A-C-C-A-A-A-T

Block ends opposite T,G & C with ddATP,ddGTP,ddCTP
(shown in bold), remove ddNTPs, then add tagged ddTTP.

○-----T-A-C T-A-T-G-G-T-T-T-A
-----A-T-G-A-T-A-C-C-A-A-A-T
○-----T-A C-T-A-T-G-G-T-T-T-A
-----A-T-G-A-T-A-C-C-A-A-A-T
○-----T-A-C T-A-T-G-G-T-T-T-A
-----A-T-G-A-T-A-C-C-A-A-A-T
○-----T-A-C-T-A T G-G-T-T-T-A
-----A-T-G-A-T-A-C-C-A-A-A-T
○-----T-A-C-T-A T-G-G-T-T-T-A
-----A-T-G-A-T-A-C-C-A-A-A-T
○-----T-A-C-T-A-T G G-T-T-T-A
-----A-T-G-A-T-A-C-C-A-A-A-T

○-----T-A-C-T-A-T-G G-T-T-T-A
-----A-T-G-A-T-A-C-C-A-A-A-T
○-----T-A-C-T-A-T-G-G T-T-T-A
-----A-T-G-A-T-A-C-C-A-A-A-T
○-----T-A-C-T-A-T-G-G-T-T-T A
-----A-T-G-A-T-A-C-C-A-A-A-T
○-----T-A-C-T-A-T-G-G-T-T-T A
-----A-T-G-A-T-A-C-C-A-A-A-T
○-----T-A-C-T-A-T-G-G-T-T-T
-----A-T-G-A-T-A-C-C-A-A-A-T

Remove all non-immobilized DNA, then release, size-separate, and detect strands with tagged terminal T.



FIG. 23

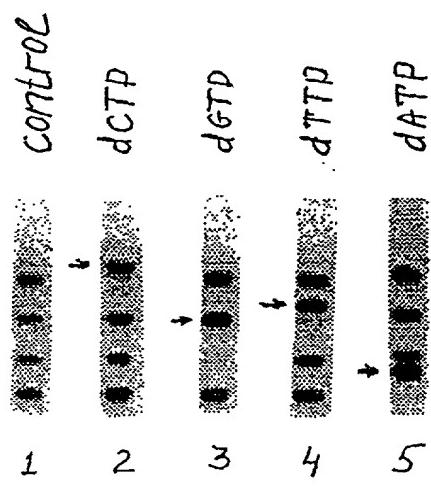


FIG. 24

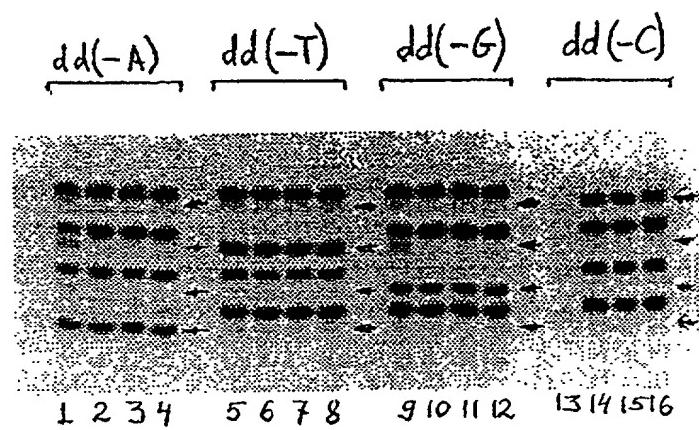


FIG. 25

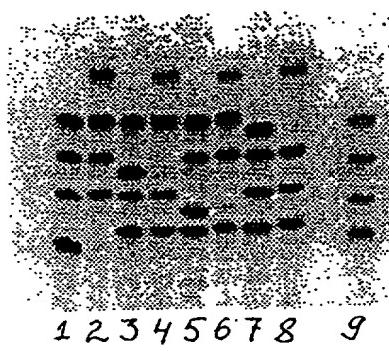


FIG. 26

Fe/EDTA DNase I

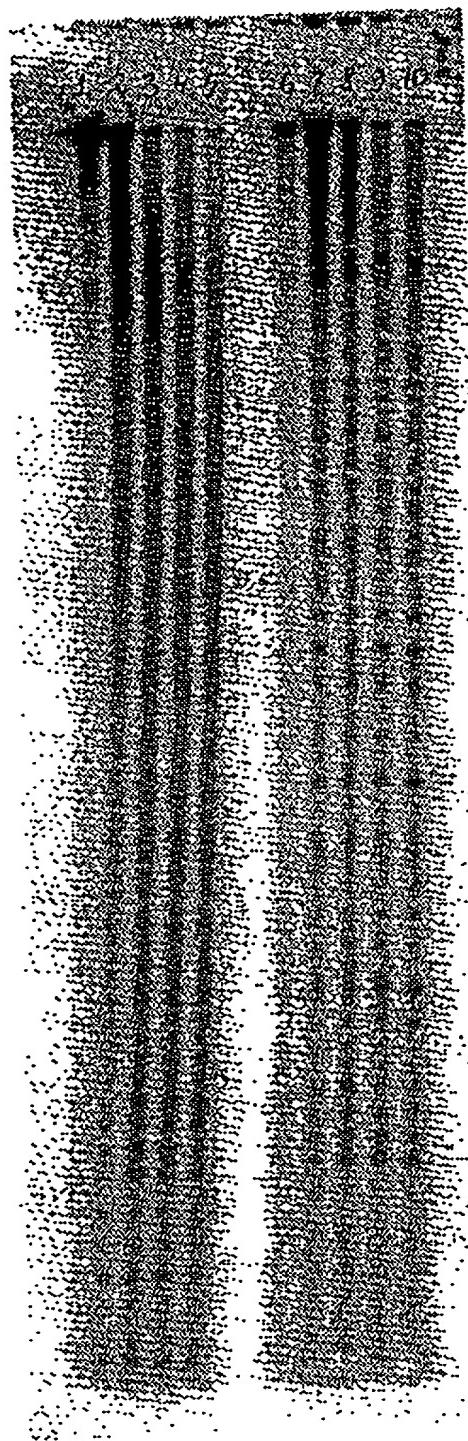
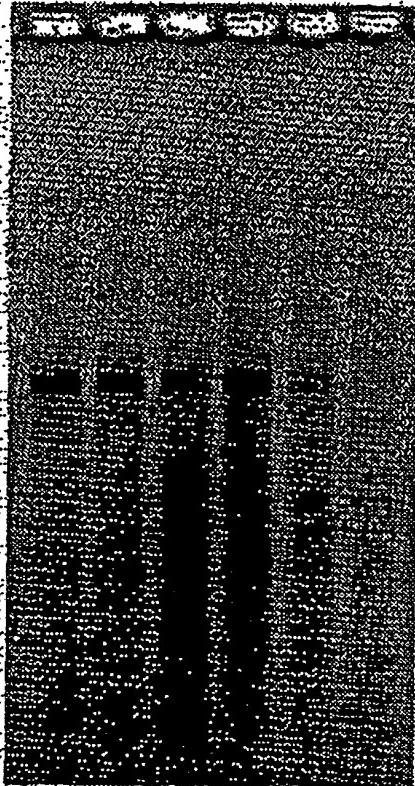


FIG. 27

EtBr staining

1 2 3 4 5 6



^{32}P -dATP

7 8 9 10 11 12

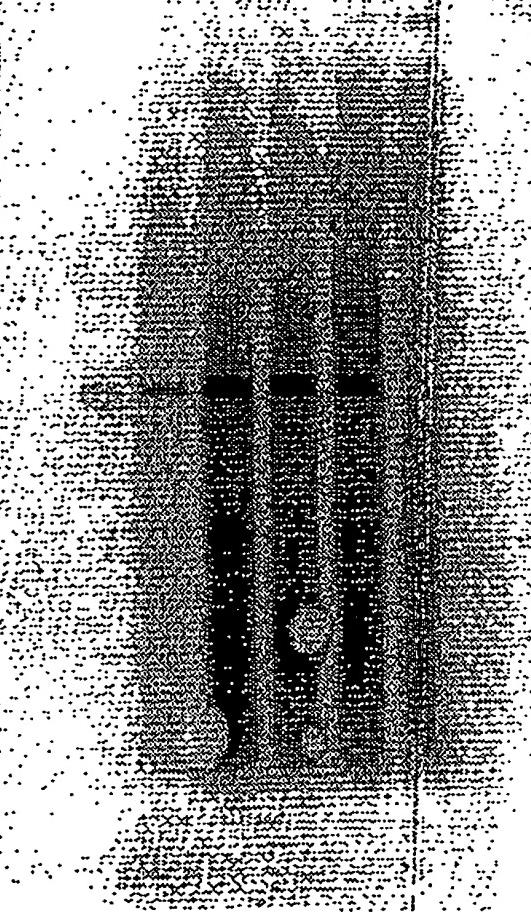


FIG. 28A

FIG. 28B

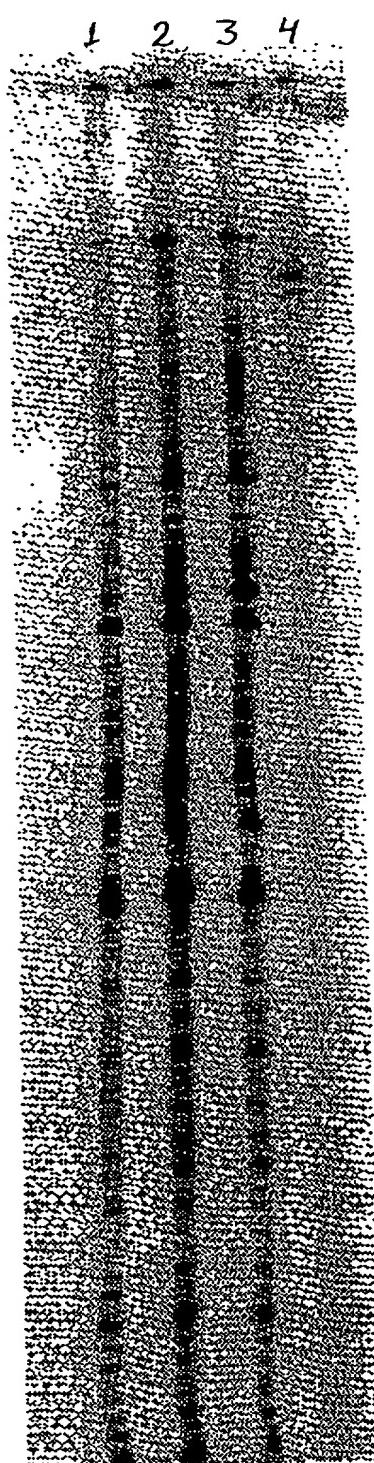
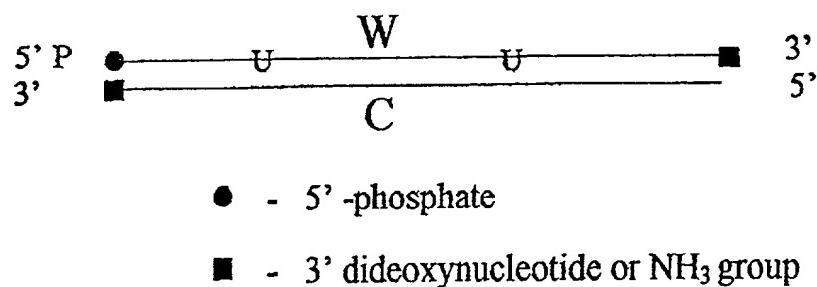


FIG. 29

FIG. 30A



| | | | | |
|----|---|-----|-------|-----------------|
| 5' | — | X | 3' OH | 4 C-X oligos |
| 5' | — | XY | 3' OH | 16 C-XY oligos |
| 5' | — | XYZ | 3' OH | 64 C-XYZ oligos |

X, Y and Z are A, T, G or C

FIG. 30B

Map of the XYZ sites

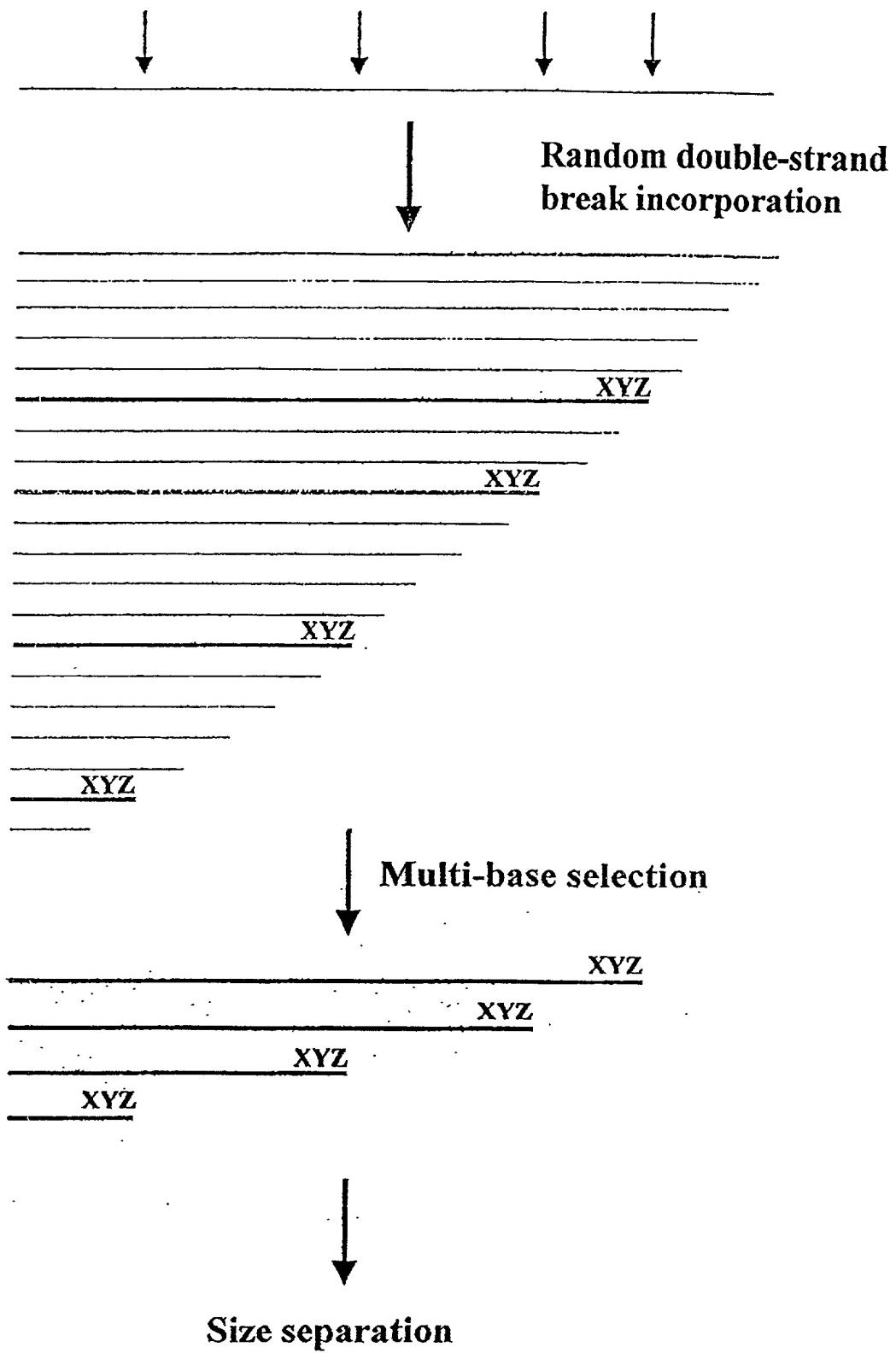


FIG. 31

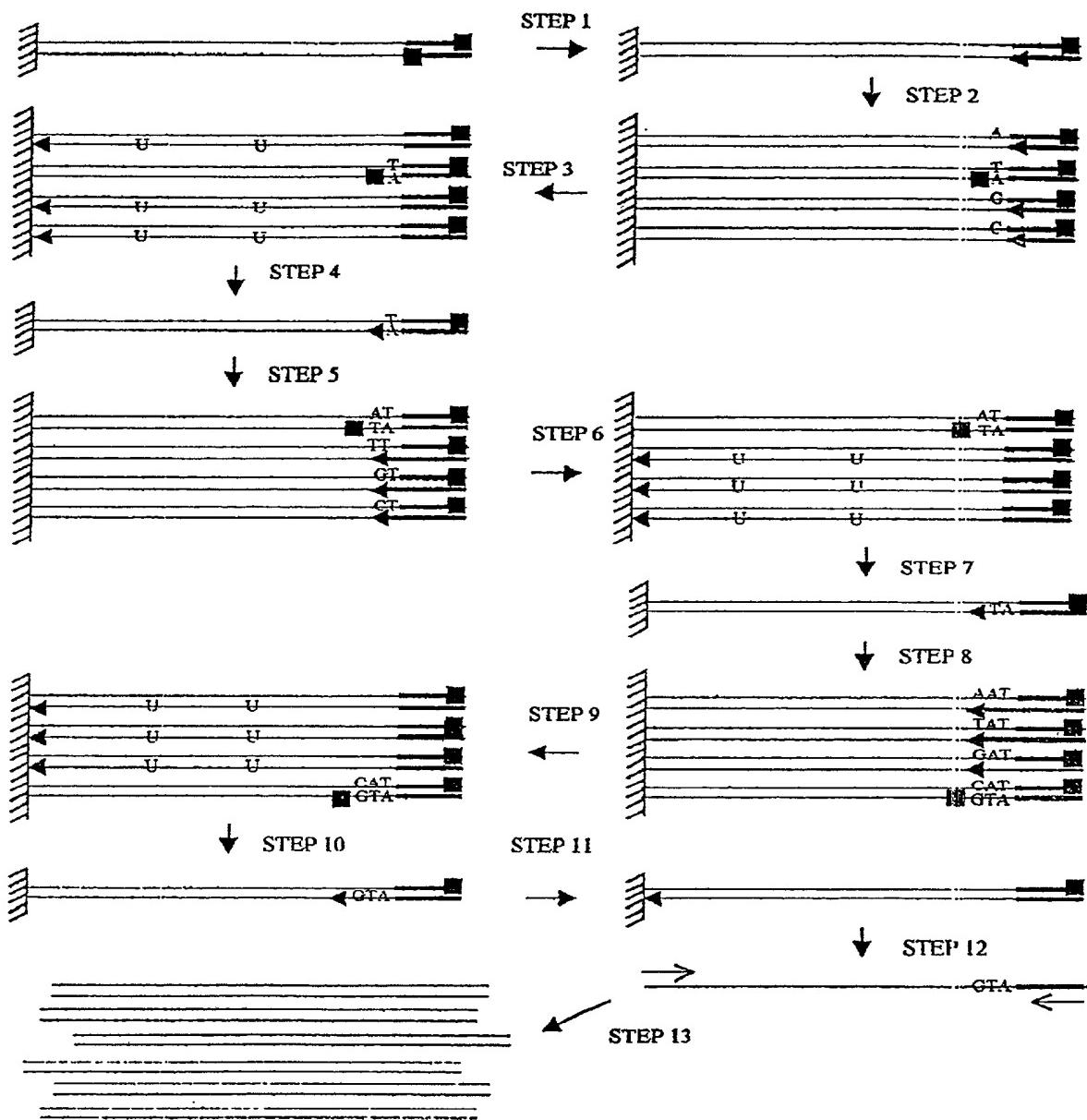


FIG. 32

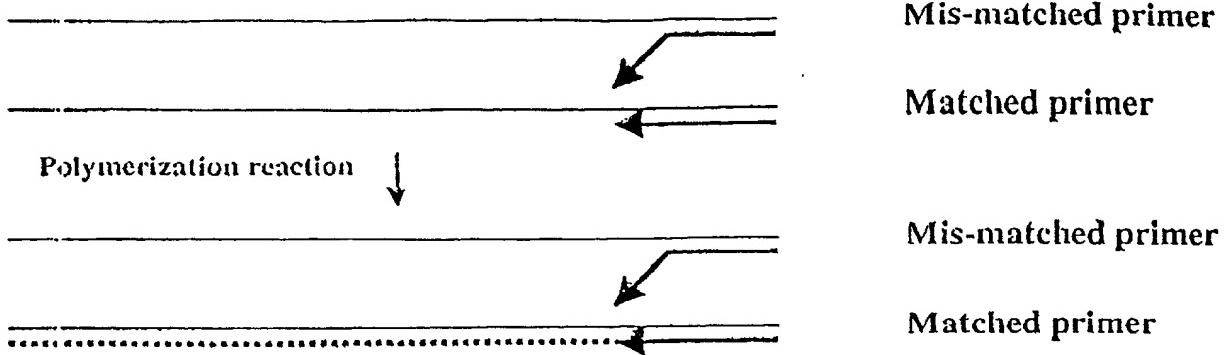


FIG. 33A

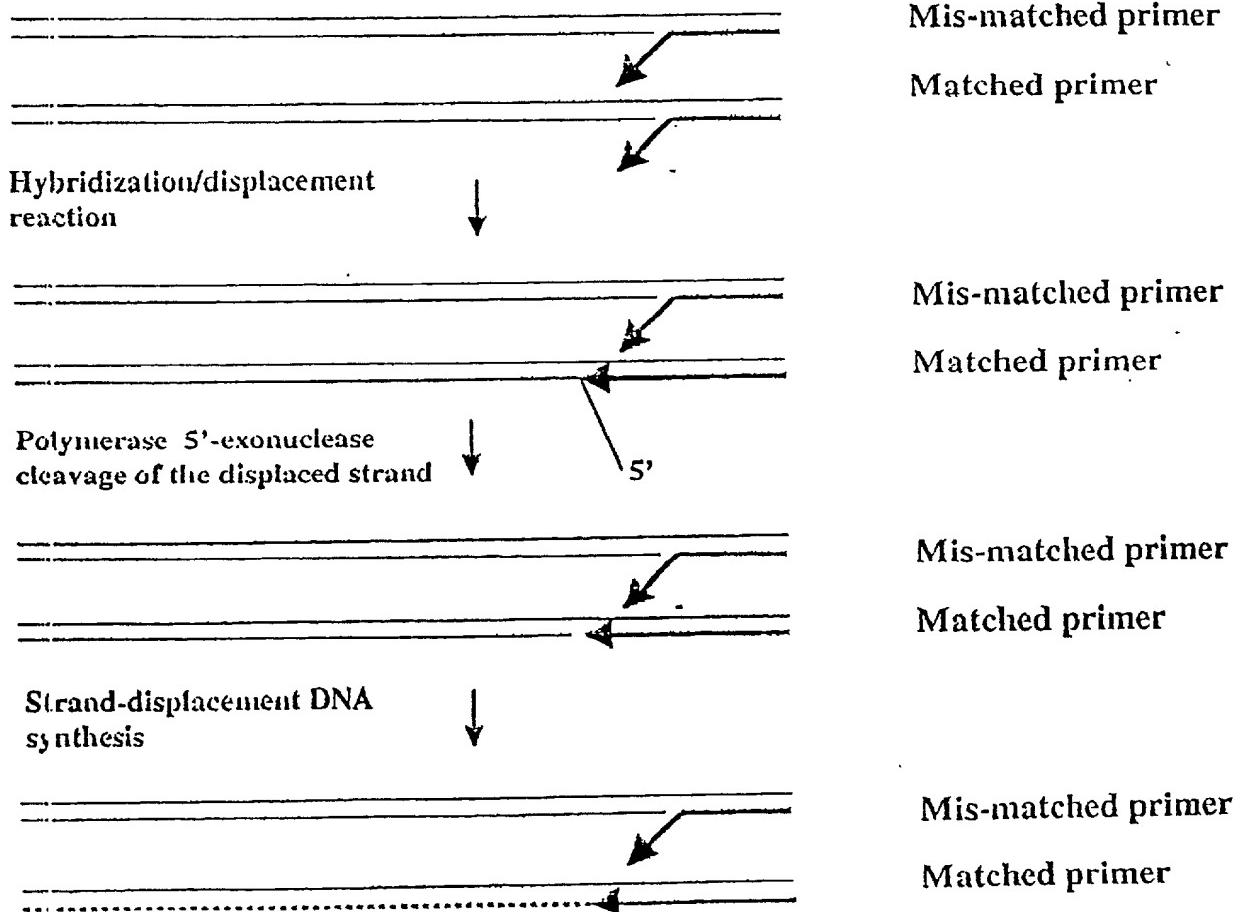


FIG. 33B